PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

WRITTEN OPINION
(PCT Rule 66)

Date of mailing *(day/month/year)*	06.06.2006
Applicant's or agent's file reference: 15658PCT00	**REPLY DUE within 0 month(s) and 15 days** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK2005/000137	28.02.2005	01.03.2004

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/24 C12Q1/04 C12Q1/34

Applicant
MYCOMETER APS et al.

1. This written opinion is the **second** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the International preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 01.07.2006

Name and mailing address of the international preliminary examining authority:
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WRITTEN OPINION International application No. PCT/DK2005/000137

I. Basis of the opinion

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-21 as originally filed

Claims, Numbers

1-47 as originally filed

Drawings, Sheets

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1 46 47
Inventive step (IS)	Claims	1-47
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION
SEPARATE SHEET

International application No. PCT/DK2005/000137

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:
D1 : WO 03/012397 A (MATSUSHITA SEIKO CO., LTD.) 13 February 2003 (2003-02-13)
D2 : US 5 811 251 A (A. HIROSE ET AL.) 22 September 1998 (1998-09-22)
D3 : EP 0 574 977 A (J. D. BERG) 22 December 1993 (1993-12-22)
D4 : US 4 871 662 A (E.ROSOV) 3 October 1989 (1989-10-03)

As D1 is written in Japanese the reasoning below and cited passages will be taken from the English language family member of D1, namely US 2004/219628, which is assumed to have the same content.

2 NOVELTY

2.1 Document D1 discloses (the references in parentheses applying to US2004/219628 as explained above) a collection unit ("a microorganism collecting chip") in which microorganisms present in a sample are trapped and subsequently detected by colour, fluorescence or luminescence, a microorganism collecting kit and a method of quantifying microorganisms using this microorganism collecting kit. (page 1, paragraph 1). The collection unit for the microorganisms includes a first filter for removal of contaminants with a pore size of 5-20 microns which allows the passage of microorganisms in the sample and a second filter with a pore size of 0.2-0.8 microns for trapping the microorganisms for detection (page 1, paragraph 5 and page 2, paragraph 31). (NB. The contaminants referred to in D1 are various types of debris which may interfere with the detection process (page 2, paragraph 30). The present application describes this type of debris as "larger particles" , whereas the contaminants are the microorganisms to be detected). The collection unit is provided with a suction filtration unit for applying negative pressure to the collection unit thereby facilitating flow of the sample through the filters (page 1, paragraphs 13-14 and page 3, paragraph 40). Quantification of microorganisms is provided by trapping said microorganisms on a filter followed by staining (page 2, paragraph 19). Alternatively quantification of the microorganisms trapped on the collection filter is

achieved by differential colouration of the various classes of microorganisms, both living and dead, by application of different colorant compounds (page 3, paragraph 45 and page 6, paragraph 79). Furthermore viable cells are detected by colouring using compounds which react with enzymes present in the microbial cells to form coloured or fluorescent products. Various examples include 4- methylumbelliferone derivatives (page 6, paragraph 81).
When a test sample is a solid sample such as foodstuffs including meat and vegetables, it is homogenised to prepare a liquid specimen (page 5, paragraph 64).

Various additives can be added to the sample liquid. Surfactants for releasing microorganisms which may be adhered to debris in the sample, polypeptone for maintaining the activity of the microorganisms or a polyhydric alcohol for preventing deactivation of the microorganisms or decay of luminescence caused by drying of the filter surface (page 7, paragraph 83).
The difference between the present application (PA) and D1 is that instead of measuring the microorganisms trapped on the filter surface by staining, the liquid vehicle surrounding the microorganisms can be used as the object for the measurement and thus a relatively simple measurement apparatus can be used which does not necessitate means for optical measurement which focus on the filter surface. As this feature is not disclosed in D1, the PA may be considered to be novel over D1.
However D3 discloses (the references in parentheses applying to this document) "a direct method for detecting very low levels of coliform contamination in products for human consumption comprising contacting the microorganisms with a methylumbelliferone substrate. The substrate is hydrolysed into methylumbelliferone by an enzyme given off by the microorganisms. The methylumbelliferone is detected by its fluorescence, either in solution or ...." (abstract). Furthermore "The general procedure for the detection of TC (Total coliform) or FC (Fecal coliform) activity ..... is as follows: (a) the sample is concentrated by passing it through a membrane filter (0.2 micrometers to 0.80 micrometers pore size);
(b) the microorganisms which are retained with the filter are aseptically placed in contact with a sterile medium containing the appropriate 4-MU-substrate; and the resulting fluorescence is measured and utilized as the rate of production of fluorescent product in the liquid medium associated with the sample determined at regular intervals over about fifteen minutes using a fluorescence detecting meter

(column 6, line 52-column 7, line 8).
Consequently the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claims 1, 46 and 47 are not new in the sense of Article 33(2) PCT.

3. INVENTIVE STEP

D3 is considered to be the closest prior art (CPA). The difference between the CPA and claim 2 of the PA is that prior to passing a contaminant (microorganism) containing medium through a filter for concentrating the contaminants on the influent side of the filter, the medium is passed through a pre-filter that does not retain the contaminants but retains larger particles. The problem to be solved is considered to be how to remove larger particles or debris which may interfere with the microorganism detection step. The solution is the incorporation of a pre-filter.
As discussed in 2.1 above, D1 discloses, inter alia, a collection unit for the microorganisms includes a first filter for removal of contaminants with a pore size of 5-20 microns which allows the passage of microorganisms in the sample and a second filter with a pore size of 0.2-0.8 microns for trapping the microorganisms for detection (page 1, paragraph 5 and page 2, paragraph 31). As this first filter has exactly the same purpose as the pre-filter of the PA it would be obvious to the man skilled in the art to combine the teachings of D3 and D1 to arrive at the solution to the problem outlined above.
Consequently the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 2 does not involve an inventive step in the sense of Article 33(3) PCT.

4. Dependent claims 3-45 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, see documents D1-D4 and the corresponding passages cited in the search report.

5. The subject matter of claims 1-47 meets the requirements of Art. 33(4) PCT, having regard to industrial application.